AF/1652

BEST AVAILABLE COPY

Docket No.: 0010-1057-0

OBLON
SPIVAK
McCLELLAND
MAIER
&
NEUSTADT
P.C.

ATTORNEYS AT LAW

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

RE: Application Serial No.: 09/441,055
Applicants: Yoshihiro USUDA, et al.
Filing Date: November 16, 1999
For: METHOD FOR PRODUCING L-METHIONINE BY FERMENTATION
Group Art Unit: 1652
Examiner: Fronda, C.L.

SIR:

Attached hereto for filing are the following papers:

Appeal Brief w/attached Appendices; Cited References (2 - Duchange, et al. and Zakin, et al.); Cited Attachment (1 - Capon v. Eshhar)

Our credit card payment form in the amount of **$500.00** is attached covering any required fees. In the event any variance exists between the amount enclosed and the Patent Office charges for filing the above-noted documents, including any fees required under 37 C.F.R 1.136 for any necessary Extension of Time to make the filing of the attached documents timely, please charge or credit the difference to our Deposit Account No. 15-0030. Further, if these papers are not considered timely filed, then a petition is hereby made under 37 C.F.R. 1.136 for the necessary extension of time. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Stephen G. Baxter
Registration No. 32,884

Vincent K. Shier, Ph.D.
Registration No. 50,552

Customer Number
22850
(703) 413-3000 (phone)
(703) 413-2220 (fax)

Docket No.: 0010-1057-0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

YOSHIHIRO USUDA, ET AL : EXAMINER: FRONDA, C. L.

SERIAL NO. : 09/441,055 :

FILED: NOVEMBER 16, 1999 : GROUP ART UNIT: 1652

FOR: METHOD FOR PRODUCING L-METHIONINE BY FERMENTATION

APPEAL BRIEF

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

SIR:

This is an appeal of the Final Rejection of Claims 31, 33, and 35 in the above-identified application set forth in the Official Action mailed November 18, 2005.

I. Real Party of Interest

The real party of interest is Ajinomoto Co., Inc., located in Tokyo, Japan, by virtue of the assignment recorded in the U.S. Patent and Trademark Office on February 16, 2000, at reel 010614, frames 0980-0982.

II. Related Appeals and Interferences

Appellants, Appellants' legal representative and their assignee are not aware of any appeals or interferences which will directly affect or be directly affected by or having a bearing on the Board's decision in this appeal.

III. Status of Claims

Claims 1-9, 11-31, 33, and 35 are the only claims pending in the above-identified application.

Claims 1-9 and 11-30 stand withdrawn.

Claims 31, 33, and 35 stand rejected.

Claims 31, 33, and 35 are appealed herein.

IV. Status of Amendments filed under 37 C.F.R. §1.116

An Amendment under 37 C.F.R. §1.116 was not filed in this application.

V. Summary of the Claimed Subject Matter

As recited in independent Claim 30, the present invention provides a method for producing L-methionine by culturing a recombinant *Escherichia* bacterium in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein the bacterium is deficient in repressor of L-methionine biosynthesis system encoded by the endogenous *metJ* gene and has L-methionine productivity, and activity of intracellular homoserine transsuccinylase encoded by the *metA* gene of a *Escherichia* bacterium is increased compared to an unmodified *Escherichia* bacterium by increasing copy number of the *metA* gene including its own promoter, or replacing the native promoter with a stronger promoter. (see the specification at page 15, lines 3-5, 9-12, and 16-21, page 6, lines 10-14, page 7, line 20 to page 8, line 6, page 10, line 19 to page 15, line 3, and page 16, line 8 to page 20, line 7)

VI. Grounds of Rejection

1. Claims 31, 33, and 35 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement.

VII. Arguments

(A) Claims 31, 33, and 35 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. This rejection is untenable and should not be sustained.

This ground of rejection largely relates to the Examiner's position that the scope of the recited *metJ*, *metA*, *metK*, *metB*, and *metL* genes are not supported by an adequate written description and encompass an undefined genus. The Examiner further alleges that the genus defined by the *metJ*, *metA*, *metK*, *metB*, and *metL* genes includes many polynucleotides with widely differing nucleotide sequences and structures, despite the fact that the claims specifically define these genes as arising from an *Escherichia* bacterium. The Examiner also disregards the fact that the sequences for the *metA* (see SEQ ID NOs: 25 and 26) and *metK* (see SEQ ID NOs: 17 and 18) genes from *E. coli* are provided in the Sequence Listing for the present application and that two references are provided evidencing that *Escherichia* sequence for the *metB*, *metJ*, and *metL* sequences were published in 1983.

Appellants disagree with the foregoing assertions and conclusions by the Examiner. In fact, Appellants submit that the *metJ* and *metK* genes are "endogenous" genes to the *Escherichia* bacterium, while the *metA*, *metB*, and *metL* genes are derived from a *Escherichia* bacterium. With respect to the term "endogenous," Appellants remind the Office that the specification and the list therein of possible sources of endogenous genes need not even be given consideration. As even a cursory review of the claimed invention reveals the present

method is one relating to a recombinant *Escherichia* bacterium and, thus, the term endogenous would refer to the recombinant *Escherichia* bacterium.

Moreover, Appellants note that the sequences for the *metA* (see SEQ ID NOs: 25 and 26) and *metK* (see SEQ ID NOs: 17 and 18) genes are provided in the Sequence Listing for the present application. Further, the nucleotide sequence for *metB* and *metJ* are described in Duchange et al, J. Biol. Chem., 258, 14868-14871 (1983), which is cited on page 12, lines 21-25 of the present specification (copy **enclosed herewith**). The nucleotide sequence for the *metL* gene is disclosed in Zakin et al, J. Biol. Chem., 258, 3028-3031 (1983), a copy of which is **enclosed herewith**. Therefore, the endogenous *metJ* and *metK* and the *metA*, *metB*, and *metL* of an *Escherichia* bacterium are fully supported by the specification and the general knowledge in the art as of the present invention.

Indeed, the Courts have recently held that the "written description" requirement must be applied in the context of the particular invention and the state of the knowledge in the art (*Capon v. Eshhar*, 418 F.3d 1349, 76 USPQ2d 1078 (Fed. Cir. 2005)). In *Capon*, the Court held that the Board erred in holding that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known. The *Capon* Court further stated that when the prior art includes the nucleotide information, precedent does not set a *per se* rule that the information must be determined afresh. Therefore, where a person experienced in the field of this invention would know that the DNA of the claims is well-known, there is no requirement to once again set forth these sequences.

Further, as in *Capon*, the present invention is not in discovering which DNA sequences correspond to the various recited genes, for that is in the prior art or in the specification as filed. Instead the present invention lies in the carefully orchestrated expression of the recited genes to achieve a novel result.

The "written description" requirement states that Applicants must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution. The present invention does not concern the discovery of gene function or structure, as in *Lilly*, which as in *Capon* is improperly relied upon by the Examiner in the present application. The genes recited in the claims correspond to those that are either disclosed in the specification or are DNA sequences of known structure and function. As such, this ground of criticism with respect to written description is not appropriate for the reasons promulgated by the *Capon* Court and should be withdrawn.

The secondary source of criticism raised by the Examiner is that the claims recite "gene elements, which are not described by the specification." The Examiner alleges that gene elements that are "not particularly described" include "regulatory elements and untranslated regions." The Examiner further states that "there is no known or disclosed correlation between the coding region of a polynucleotide encoding each of the recited [genes] and the structure of the non-described regulatory elements and untranslated regions of the gene." Appellants submit that this assertion by the Examiner is misplaced and is not based or supported by any scientific fact.

The genes of the present invention relate to bacterial gene sequences derived from the genus *Escherichia*. Since these sequences are bacterial in origin, there are no intron and exons to be concerned with as there would be with genes of higher eukaryotes. Therefore, in the genes of the present invention the coding frame would be read through starting from the start codon and ending at the first stop codon. In addition, the claims specifically recite the relationship between the promoter sequence and the gene sequence, the placement of which with respect to the start codon can be determined by looking at any introductory genetics text.

Appellants again submit that the novelty of the present invention does not lie in the sequences of the various recited genes, but rather in the interplay between the same to bring about a novel effect. Further, the sequences corresponding to the *Escherichia* genes that are set forth in the claims of the present application are either disclosed in the specification or are known to the skilled artisan (see Duchange et al and Zakin et al). Therefore, as in *Capon*, the sequences corresponding to the genes (including the coding frame) of the present invention are known to the skilled artisan when the specification of the present application is read together with the knowledge available in the art at the time of the present invention. Therefore, the claims under appeal do comply with the written description requirement of 35 U.S.C. §112, first paragraph.

In view of the foregoing, it is respectfully requested that this rejection be REVERSED.

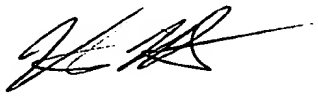
VIII. CONCLUSION

For the above reasons, Claims 31, 33, and 35 are not unpatentable under 35 U.S.C. §112, first paragraph, as these claims fully comply with the written description requirement.

Therefore, the Examiner's rejections should be REVERSED.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Stephen G. Baxter, Ph.D.
Registration No. 32,884

Vincent K. Shier, Ph.D.
Registration No.50,552

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413-2220
(OSMMN 08/03)

Attachments: Claims Appendix: Pending Claims in U.S. Application Serial No. 09/441,055
Evidence Appendix
1.) Duchange et al, J. Biol. Chem., 258, 14868-14871 (1983).
2.) Zakin et al, J. Biol. Chem., 258, 3028-3031 (1983).
Related Proceedings Appendix

Capon v. Eshhar, 418 F.3d 1349, 76 USPQ2d 1078 (Fed. Cir. 2005).

CLAIMS APPENDIX

Pending Claims in U.S. Application Serial No. 09/441,055

1. (Withdrawn) A microorganism which is deficient in repressor of L-methionine biosynthesis system and the L-methionine productivity.

2. (Withdrawn) A microorganism having enhanced intracellular homoserine transsuccinylase activity and L-methionine productivity.

3. (Withdrawn) A microorganism which is deficient in repressor of L-methionine biosynthesis system, and has enhanced intracellular homoserine transsuccinylase activity and L-methionine productivity.

4. (Withdrawn) The microorganism according to any one of claims 1 to 3, which further exhibits reduced intracellular S-adenosylmethionine synthetase activity.

5. (Withdrawn) The microorganism according to any one of claims 2 to 4, wherein the enhanced homoserine transsuccinylase activity is obtained by increasing copy number of a gene coding for the intracellular homoserine transsuccinylase, or enhancing an expression regulatory sequence for the gene.

6. (Withdrawn) The microorganism according to any one of claims 1 to 4, which has homoserine transsuccinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized.

7. (Withdrawn) The microorganism according to any one of claims 1 to 6, which exhibits L-threonine auxotrophy.

8. (Withdrawn) The microorganism according to any one of claims 1 to 7, which exhibits enhanced intracellular cystathionine γ-synthase activity and enhanced intracellular aspartokinase-homoserine dehydrogenase II activity.

9. (Withdrawn) The microorganism according to any one of claims 1 to 8, which belongs to the genus *Escherichia.*

10. (Canceled)

11. (Withdrawn) A DNA which codes for homoserine transsuccinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized, wherein the homoserine transsuccinylase has the amino acid sequence of SEQ ID NO: 26 including a mutation corresponding to replacement of arginine by cysteine at the 27th position, mutation corresponding to replacement of isoleucine by serine at the 296th position, mutation corresponding to replacement of proline by leucine t the 298th position, mutation corresponding to replacement of arginine by cysteine at the 27th position and replacement of isoleucine by serine at the 296th position, mutation corresponding to replacement of isoleucine by serine at the 296th position and replacement of proline by leucine t the 298th position, mutation corresponding to replacement of proline by leucine t the 298th position and replacement of arginine by cysteine at the 27th position, or mutation corresponding to replacement of arginine by cysteine at the 27th position, replacement of isoleucine by serine

at the 296th position, and replacement of proline by leucine t the 298th position.

12. (Withdrawn) A method for producing L-methionine which comprises culturing a microorganism in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein the microorganism is deficient in a repressor of L-methionine biosynthesis system and has L-methionine productivity.

13. (Withdrawn) The method according to Claim 12, wherein the microorganism further comprises at least one characteristic selected from the group consisting of:

(a) exhibits reduced intracellular S-adenosylmethionine synthetase activity;

(b) exhibits L-threonine auxotrophy;

(c) exhibits enhanced intracellular cystathionine γ-synthase activity and enhanced intracellular aspartokinase homoserine dehydrogenase II activity; and

(d) has a homoserine transsuccinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized.

14. (Withdrawn) The method according to Claim 12, wherein the microorganism is an *Escherichia* bacterium.

15. (Withdrawn) The method according to Claim 12, wherein the microorganism is *Escherichia coli*.

16. (Withdrawn) The method of Claim 12, wherein the repressor of L-methionine biosynthesis is the metJ protein.

17. (Withdrawn) The method of Claim 13, wherein the S-adenosylmethionine synthetase is encoded by the metK gene.

18. (Withdrawn) The method of Claim 13, wherein the cystathionine γ-synthase is encoded by the metB gene.

19. (Withdrawn) The method of Claim 13, wherein the aspartokinase homoserine dehydrogenase II is encoded by the metL gene.

20. (Withdrawn) The method of Claim 13, wherein the homoserine transsuccinylase comprises the amino acid sequence of SEQ ID NO:26, wherein at amino acid number 27 the arginine is replaced with an cysteine, at amino acid number 296 the isoleucine is replace with a serine, and at amino acid number 298 the proline is replaced with a leucine.

21. (Withdrawn) A method for producing L-methionine which comprises culturing a microorganism in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein the microorganism has enhanced intracellular homoserine transsuccinylase activity and L-methionine productivity.

22. (Withdrawn) The method according to Claim 21, wherein the enhanced homoserine transsuccinylase activity is obtained by increasing the copy number of a gene coding for the intracellular homoserine transsuccinylase, or enhancing an expression regulatory sequence for the gene.

23. (Withdrawn) The method according to Claim 21, wherein the microorganism further comprises at least one characteristic selected from the group consisting of:

(a) exhibits reduced intracellular S-adenosylmethionine synthetase activity;

(b) exhibits L-threonine auxotrophy;

(c) exhibits enhanced intracellular cystathionine γ-synthase activity and enhanced intracellular aspartokinase-homoserine dehydrogenase II activity; and

(d) has a homoserine transsuccinylase for which concerted inhibition by L-methionine and s-adenosylmethionine is desensitized.

24. (Withdrawn) The method according to Claim 21, wherein the microorganism is an *Escherichia* bacterium.

25. (Withdrawn) The method according to Claim 21, wherein the microorganism is *Escherichia coli*.

26. (Withdrawn) The method of Claim 21, wherein the repressor of L-methionine biosynthesis is the metJ protein.

27. (Withdrawn) The method of Claim 22, wherein the S-adenosylmethionine synthetase is encoded by the metK gene.

28. (Withdrawn) The method of Claim 22, wherein the cystathionine γ-synthase is encoded by the metB gene.

29. (Withdrawn) The method of Claim 22, wherein the aspartokinase homoserine dehydrogenase II is encoded by the metL gene.

30. (Withdrawn) The method of Claim 22, wherein the homoserine transsuccinylase comprises the amino acid sequence of SEQ ID NO:26, wherein at amino acid number 27 the arginine is replaced with an cysteine, at amino acid number 296 the isoleucine is replace with a serine, and at amino acid number 298 the proline is replaced with a leucine.

31. (Previously Presented) A method for producing L-methionine which comprises culturing a recombinant *Escherichia* bacterium in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein the bacterium is deficient in repressor of L-methionine biosynthesis system encoded by the endogenous *metJ* gene and has L-methionine productivity, and activity of intracellular homoserine transsuccinylase encoded by the *metA* gene of a *Escherichia* bacterium is increased compared to an unmodified *Escherichia* bacterium by increasing copy number of the *metA* gene including its own promoter, or replacing the native promoter with a stronger promoter.

32. (Canceled)

33. (Previously Presented) The method according to claim 31, wherein the bacterium further comprises at least one characteristic selected from the group consisting of:

(a) exhibits reduced activity of intracellular S-adenosylmethionine synthetase encoded by the endogenous *metK* gene as compared to an unmodified *Escherichia* bacterium;

(b) exhibits L-threonine auxotrophy;

(c) exhibits enhanced activity of intracellular cystathionine γ-synthase encoded by the *metB* gene of a *Escherichia* bacterium and enhanced activity of intracellular aspartokinase-homoserine dehydrogenase II encoded by the *metL* gene of a *Escherichia* bacterium is increased compared to an unmodified *Escherichia* bacterium by increasing copy number of each of the genes including their own promoters, or replacing the native promoter with a stronger promoter; and

(d) has a homoserine transsucinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized, wherein the homoserine transsuccinylase comprising the amino acid sequence of SEQ ID NO: 26 contains at least one amino acid replacement wherein said at least one amino acid replacement is independently selected from the group consisting of replacement of the amino acid residue Arg-27 with cysteine, replacement of the amino acid residue Ile-296 with serine, and replacement of the amino acid residue Pro-298 with leucine.

34. (Canceled)

35. (Previously Presented) The method according to Claim 31, wherein the bacterium is *Escherichia coli*.

36. – 40. (Canceled)

EVIDENCE APPENDIX

1.) Duchange et al, J. Biol. Chem., 258, 14868-14871 (1983).

2.) Zakin et al, J. Biol. Chem., 258, 3028-3031 (1983).

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 258, No. 5, Issue of March 10, pp. 3028–3031, 1983
Printed in U.S.A.

Nucleotide Sequence of the *metL* Gene of *Escherichia coli*

ITS PRODUCT, THE BIFUNCTIONAL ASPARTOKINASE II-HOMOSERINE DEHYDROGENASE II, AND THE BIFUNCTIONAL PRODUCT OF THE *thrA* GENE, ASPARTOKINASE I-HOMOSERINE DEHYDROGENASE I, DERIVE FROM A COMMON ANCESTOR*

(Received for publication, August 9, 1982)

Mario M. Zakin‡, Nathalie Duchange, Pascual Ferrara§, and Georges N. Cohen

From the Unité de Biochimie Cellulaire, Département de Biochimie et Génétique Moléculaire, Institut Pasteur, 28 Rue du Dr. Roux 75724 Paris, Cedex 15, France

The total nucleotide sequence (2427 nucleotides) of the *metL* gene of *Escherichia coli* coding for the dimeric aspartokinase II-homoserine dehydrogenase II (809 amino acid residues, M_r = 88,726/chain) is presented. Comparison of the translated sequence with that of aspartokinase I-homoserine dehydrogenase I, the product of the *thrA* gene of the same organism, shows that the two proteins are highly homologous and that they derive from a common ancestor.

In *Escherichia coli* K12, the gene *metL* codes for one of the enzymes involved in methionine biosynthesis, aspartokinase (EC 2.7.2.4) II-homoserine dehydrogenase (EC 1.1.1.3) II (1). It is a bifunctional enzyme, whose synthesis is controlled by the concentration of methionine in the intracellular pool (1). It catalyzes together with another bifunctional protein, aspartokinase I-homoserine dehydrogenase I and with aspartokinase III, two steps in the common biosynthetic pathway which leads from aspartate to diaminopimelate and lysine, to methionine, and to threonine and isoleucine. The two *E. coli* iso- and bifunctional enzymes, aspartokinase II-homoserine dehydrogenase II and aspartokinase I-homoserine dehydrogenase I indeed possess remarkable similarities (1); when they are compared using immunochemical techniques, a cross-reaction is obtained when denatured immunogens and antigens are used. These results suggest that aspartokinase I-homoserine dehydrogenase I and aspartokinase II-homoserine dehydrogenase II possess some homologous amino acid sequences and that they are evolutionally related (2).

Since the DNA sequence of the *thrA* gene and the amino acid sequence of its product, aspartokinase I-homoserine dehydrogenase I, have been determined recently (3), we decided to study the DNA sequence of the *metL* gene and the amino acid sequence of its product, aspartokinase II-homoserine dehydrogenase II, in order to compare the two structures.

The gene *metL*, together with *metJ*, a pleiotropic regulatory gene and with two other structural genes, *metB* and *metF*, coding for γ-cystathionine synthetase and 5–10 methylenetetrahydrofolate reductase, respectively, are clustered at position 88 min of the *E. coli* K12 circular chromosome (4). We have previously cloned the *metJBLF* gene cluster in the plasmid pBR322 and have localized precisely the origin of *metL* by its DNA sequence (5). In this work, we present the complete DNA sequence of the *metL* gene and the comparison between the amino acid sequence of its product, aspartokinase II-homoserine dehydrogenase II, and the amino acid sequence of the product of *thrA*, aspartokinase I-homoserine dehydrogenase I.

* This work was supported by the Centre National de la Recherche Scientifique and the Délégation Générale á la Recherche Scientifique et Technique. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom all correspondence should be addressed.

§ Recipient of an Association pour le Développement de l'Institut Pasteur Fellowship when this work was performed.

MATERIALS AND METHODS

Molecular Cloning of the metL Gene—The pBR322 hybrid plasmid containing the *metB*, *metL*, and *metF* genes (pMAD4) was constructed as previously described (5).

Enzymes and Materials—DNA polymerase large fragment and different restriction endonucleases were obtained from New England Biolabs. Acrylamide was from BDH (Poole, England), urea and boric acid from SERVA (Heidelberg, West Germany), hydrazine from Eastman, and dimethyl sulfate was from Aldrich. All other chemicals were analytical grade or purer, mostly from Merck. Phenol was distilled.

Nucleotide Sequence Determinations—The nucleotide sequences were determined by the chemical method of Maxam and Gilbert (6).

RESULTS AND DISCUSSION

Nucleotide Sequence of the metL Gene—A restriction fragment isolated from a λh80 transducing bacteriophage by cleavage with *Eco*RI and *Pst*I was cloned in pBR322 (5). Genetical analysis showed that this fragment carries the *metB*, *metL*, and *metF* genes. The origin of the *metL* gene was previously localized by DNA sequencing (5). Translation of the obtained sequence yields the NH_2-terminal nonapeptide of aspartokinase II-homoserine dehydrogenase II previously detected by amino acid sequence experiments (the NH_2-terminal methionine is absent from the translated product) (7). The complete nucleotide sequence of the *metL* gene is presented here (Fig. 1). The DNA fragments and the restriction sites used in the sequence determination are indicated in Fig. 2. The gene is 2427 nucleotides long and encodes a single polypeptide chain of 809 amino acids. The protein is therefore a dimer of an identical polypeptide chain of M_r = 88,726. Except for minor discrepancies, the overall amino acid composition of the chain, the COOH-terminal residues, and the structure of some predicted cysteine-containing tryptic peptides scattered all along the sequence are in good agreement with some previous data obtained with aspartokinase II-homoserine dehydrogenase II (7) (Tables I and II).

The codon usage in *metL* gene is found to be highly nonrandom. The overall codon usage is similar to that found for example in the *trpA* gene (8) or in the *thrA* gene (3).

Comparison between the Amino Acid Sequences of Aspartokinase I-Homoserine Dehydrogenase I and Aspartokinase II-Homoserine Dehydrogenase II—Fig. 3 compares the pre-

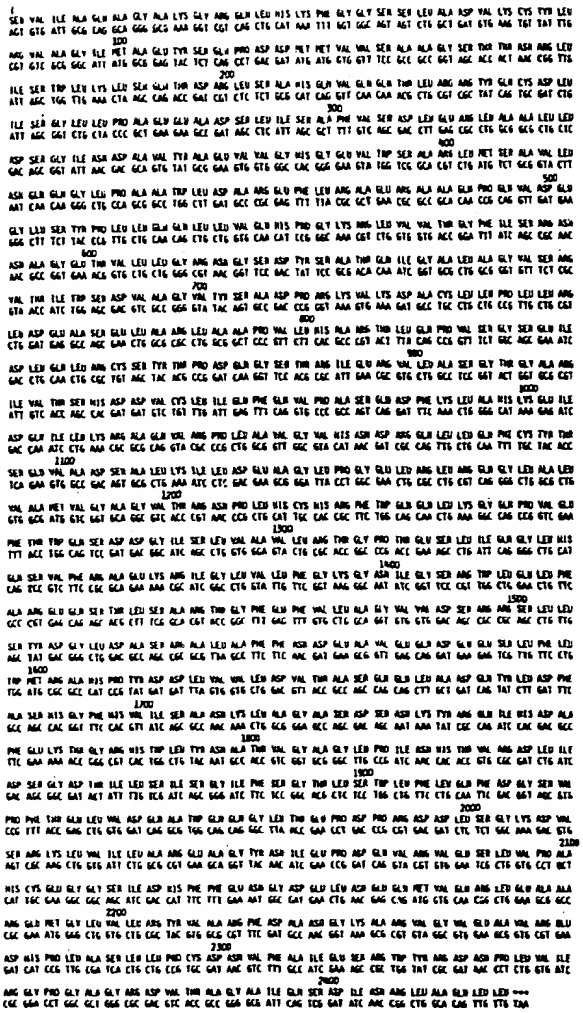


FIG. 1. **Sequence of *met*L gene.** The given sequence is that of the noncoding strand. The deduced amino acid sequence for the correct reading frame is shown.

dicted amino acid sequences of the two proteins. Since the number of residues to compare is different (809 for aspartokinase II-homoserine dehydrogenase II and 820 for aspartokinase I-homoserine dehydrogenase I), and since gaps were introduced to maximize identities, it was necessary to consider 840 positions. The PEP-program of Intelligenetics and several programs from the Centre de Calcul de l'Institut Pasteur were used in the comparison. Out of these 840 positions, 262 residues were identical, *i.e.* 31%. If one examines the amino acids which could be derived one from another by a single base change, this proportion rises to 41%, whereas, if accepted amino acid replacements (9) are considered, it rises to 39%. If both the single base changes and accepted replacements are taken into account together with the identities (when a single

TABLE I

Amino acid composition and NH_2-terminal and COOH-terminal sequence of aspartokinase II-homoserine dehydrogenase II

Amino acids	Found[a]	Predicted (from nucleotide sequence)
Lysine	23	20
Histidine	19	20
Arginine	58–59	58
Cysteine	9–10	9
Aspartate (Asx)	73	78
Threonine	26	29
Serine	60	63
Glutamate (Glx)	93	91
Proline	19–20	28
Glycine	58	63
Alanine	82	83
Valine	65	66
Methionine	8–9	8
Isoleucine	37	34
Leucine	100	102
Tyrosine	19–20	17
Phenylalanine	27	28
Tryptophan	16	12
NH_2-terminal sequence	Ser-Val-Ile-Ala-Gln-Ala-Gly-Ala-Lys-	Met-Ser-Val-Ile-Ala-Gln-Ala-Gly-Ala-Lys-
COOH-terminal sequence	Leu-Leu	Leu-Leu

[a] See Ref. 7.

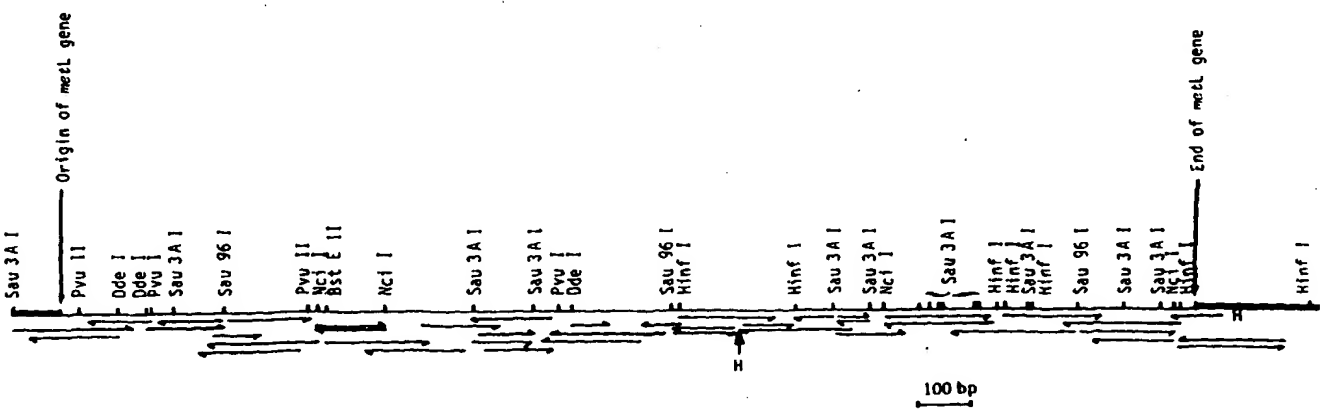


FIG. 2. **Restriction map of part of the pBR322-*met* (pMAD4) hybrid plasmid (5) corresponding to the *met*L gene, and its sequencing strategy.** The *arrows* indicate the sites used for labeling as well as the direction and extent of the sequence. *H* indicates some of the *Hpa*II sites of the gene, used for labeling and sequencing. More than 90% of the sequence has been established on both strands.

TABLE II

Amino acid composition and NH_2-terminal residues of some cysteine-containing peptides of aspartokinase II-homoserine dehydrogenase II

Amino acids	Peptide							
	Found Cys(Cm)1[a]	Predicted[b]	Found Cys(Cm)2[a]	Predicted[c]	Found Cys(Cm)6[a]	Predicted[d]	Found Cys(Cm)B[a]	Predicted[e]
Lysine			ND[f]					
Histidine			ND	2			2	2
Arginine	1	1	ND	1	1	1	1	1
Cysteine	1	1	1	1	1	1	1	1
Aspartate	3	3	3	4	1	1	1	1
Threonine					2	2		
Serine	3	4	2	2	2	2		
Glutamate	4	4	6–7	7	1	1		
Proline	1	1		1	2	1	1	1
Glycine	1	1	4	2	1	1		
Alanine	3[g]	3	1	1				
Valine	1	1	2	3				
Methionine				1				
Isoleucine	1	2	1	1				
Leucine	4–5	5	2	2			1	1
Tyrosine	1	1			1	1		
Phenylalanine	1	1	2	2				
NH_2-terminal residue	Tyr	Tyr	Val	Val	?	Cys	Asx	Asn

[a] Carboxymethyl cysteine-containing peptides are named according to Ref. 7.
[b] Corresponds in the nucleotide sequence to positions 238–321.
[c] Corresponds in the nucleotide sequence to positions 2080–2172.
[d] Corresponds in the nucleotide sequence to positions 856–888.
[e] Corresponds in the nucleotide sequence to positions 1207–1227.
[f] ND, not determined.
[g] L. Sibilli-Weill and A. Dautry-Varsat, personal communication.

base change leads to an accepted replacement, it has been scored only once) the homology between the two proteins is 46%.

The hypothesis that the two proteins were evolutionally related had been put forward by us (2) on the basis of immunochemical studies. The present results lead to the compelling conclusion that the two polypeptides derive indeed from a common ancestor.

Computer Predicted Secondary Structure of Aspartokinase II-Homoserine Dehydrogenase II—The analysis, performed according to Garnier *et al.* (10) gave 32% of helical structures, 32% of β-sheets, and 36% of other structures (coils and turns). Comparison of the theoretical structures with those of aspartokinase I-homoserine dehydrogenase I (results not shown) did not lead to easily recognizable common features. The most salient observation is that aspartokinase II-homoserine dehydrogenase II may contain less helical structures than aspartokinase I-homoserine dehydrogenase I (52%) (3). Although the predictive methods might be valuable, they can not be absolutely reliable and this analysis has been done because any similitude between otherwise homologous proteins would have been of comparative value. Only the examination of the tridimensional structures, when available, will answer the question whether the homology observed in the sequence extends to the secondary and tertiary structures.

Detailed Comparison between the Two Amino Acid and Nucleotide Sequences—Results obtained with aspartokinase I-homoserine dehydrogenase I, using limited proteolysis and a fragment derived from a non-sense mutant (11) have allowed us to divide this protein into three functional domains: an aspartokinase domain (residues 1 to approximately 249), an interface domain (residues 250 to approximately 470), and a homoserine dehydrogenase domain (residues 471 to 820).[1]

On the other hand, two dimeric proteolytic fragments have been isolated from aspartokinase II-homoserine dehydrogenase II (12, 13). One of them, endowed with homoserine dehydrogenase activity, is COOH-terminal and has a molecular weight of 74,000 (approximately 350 residues/chain). If one assumes that aspartokinase II-homoserine dehydrogenase II has a comparable triglobular structure, one can compare the distributions of homologies between the corresponding regions. This is shown in Table III, in comparison with the overall homology.

Table III shows that the homology extends throughout the two proteins confirming our immunochemical results (12), with a maximum in the dehydrogenase regions and a minimum in the middle regions. The homologies of the kinase regions are of the same order as those of the entire proteins. Among the 262 identities between the two proteins, only 100 amino acid residues correspond to identical codons. The remaining 162 residues (62%) correspond to different codons, showing that the selective pressure has been operative in order to conserve a given amino acid and that, in this case, the silent mutations have not been counterselected. This observation suggests that the divergence is an ancient event, in accordance with the fact that all *Enterobacteriaceae* tested possess the isofunctional aspartokinase and homoserine dehydrogenase activities, in contrast to many other genera (14).

The hypothesis that the aspartokinase-homoserine dehydrogenases arise from a gene fusion has repeatedly been proposed (11, 13). The fusion between ancestral genes could have preceded the duplication of the fused genes; alternatively, the individual ancestral kinase and dehydrogenase genes could have duplicated and two separate fusions would then have taken place. Unfortunately, our results do not permit a decision between the two possibilities.

In any case, the results presented here adduce unequivocal evidence that the two iso- and bifunctional aspartokinase-homoserine dehydrogenases from *E. coli* derive from a common ancestor.

[1] Fazel, A., Müller, K., Le Bras, G., Garel, J. R., Véron, M., and Cohen G. N. (1982) *Biochemistry* **21**, in press.

FIG. 3. **Comparison between the amino acid sequences of aspartokinase I-homoserine dehydrogenase I and aspartokinase II-homoserine dehydrogenase II of *E. coli*.** *Top line*, aspartokinase I-homoserine dehydrogenase I; *bottom line*, aspartokinase II-homoserine dehydrogenase II. Identical residues have been *boxed*. Homologies resulting from a single base change are marked by a *black dot*; those corresponding to an accepted amino acid substitution are indicated by a *cross*. In the sequence of aspartokinase I-homoserine dehydrogenase I, the Leu in position 11 (3) has been corrected to a Val.[2]

TABLE III

Homologies between the different regions of the two aspartokinase-homoserine dehydrogenases of E. coli

	Entire proteins[a]	Aspartokinase regions[a] (about 260 residues)	Middle regions[a] (about 230 residues)	Homoserine dehydrogenase regions[a] (about 350 residues)
	%			
Identity	31	32	20	38
Homologies (1 base change)	41	39	32	48
Homologies (accepted amino acid replacements[b])	39	40	28	47
Combined identities + homologies	46	44	36	53

[a] Gaps have been taken into account as in Fig. 3 (total of 840 positions has been considered). For the homologies rationale, see text.

[b] The only accepted amino acid replacements taken into account are the following: isoleucine-valine-leucine, serine-threonine, phenylalanine-tyrosine, arginine-lysine, and aspartate-glutamate.

Acknowledgments—We thank F. E. Baralle for his useful suggestions in the DNA sequence experiments, M. C. Py for the preparation of plasmid DNA, B. Caudron for his help in the computer work, J. Garnier for his help in obtaining the predicted secondary structures, and L. Boulet for typing the manuscript.

[2] P. Cossart, personal communication.

REFERENCES

1. Cohen, G. N., and Dautry-Varsat, A. (1980) in *Multifunctional Proteins* (Bisswanger, H., and Schmincke-Ott, E., eds) pp. 49–121, John Wiley and Sons, New York
2. Zakin, M. M., Garel, J.-R., Dautry-Varsat, A., Cohen, G. N., and Boulot, G. (1978) *Biochemistry* **17**, 4318–4323
3. Katinka, M., Cossart, P., Sibilli, L., Saint-Girons, I., Chalvignac, M. A., Le Bras, G., Cohen, G. N., and Yaniv, M. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 5730–5733
4. Bachmann, B. J., and Low, K. B. (1980) *Bacteriol. Rev.* **44**, 1–56
5. Zakin, M. M., Greene, R. C., Dautry-Varsat, A., Duchange, N., Ferrara, P., Py, M. C., Margarita, D., and Cohen, G. N. (1982) *Mol. Gen. Genet.* **187**, 101–106
6. Maxam, A. M., and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 560–564
7. Dautry-Varsat, A., Sibilli-Weill, L., and Cohen, G. N. (1977) *Eur. J. Biochem.* **76**, 1–6
8. Nichols, B. P., and Yanofsky, C. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5244–5248
9. Dayhoff, M. M. (1978) in *Atlas of Protein Sequence and Structure*, p. 346, National Biomedical Research Foundation, Washington
10. Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) *J. Mol. Biol.* **120**, 97–120
11. Véron, M., Falcoz-Kelly, F., and Cohen, G. N. (1972) *Eur. J. Biochem.* **28**, 520–527
12. Sibilli, L., Le Bras, G., Fazel, A., Bertrand, O., Dautry-Varsat, A., Cohen, G. N., and Zakin, M. M. (1982) *Biochem. Int.* **4**, 331–336
13. Dautry-Varsat, A., and Cohen, G. N. (1977) *J. Biol. Chem.* **252**, 7685–7689
14. Cohen, G. N., Stanier, R. Y., and Le Bras, G. (1969) *J. Bacteriol.* **99**, 791–801

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 258, No. 24, Issue of December 25, pp. 14868-14871, 1983
Printed in U.S.A.

Structure of the *metJBLF* Cluster in *Escherichia coli* K12

SEQUENCE OF THE *metB* STRUCTURAL GENE AND OF THE 5'- AND 3'-FLANKING REGIONS OF THE *metBL* OPERON*

(Received for publication, June 13, 1983)

Nathalie Duchange, Mario M. Zakin‡, Pascual Ferrara, Isabelle Saint-Girons, Insoo Park, Sy V. Tran, Marie-Claire Py, and Georges N. Cohen

From the Unité de Biochimie Cellulaire, Département de Biochimie et Génétique Moléculaire, Institut Pasteur, 75724 Paris Cedex 15, France

The total nucleotide sequence (1,158 nucleotides) of the *metB* gene of *Escherichia coli* coding for cystathionine γ-synthase (386 amino acid residues, M_r = 41,503/chain) is presented. The nucleotide sequences of the flanking regions of the *metB* and *metL* genes are also presented. Analysis of these sequences and identification of a promoter region upstream from the *metB* gene confirms that *metB* and *metL* form an operon. The transcription direction is from *metB* to *metL*; the start site of the gene transcription has been determined. There is no structural evidence of a classical attenuation mechanism in the regulation of this operon coding for enzymes implicated in an amino acid biosynthetic pathway. Finally, the overall organization of the *metJBLF* gene cluster is discussed.

In *Escherichia coli* K12 the genes involved in methionine biosynthesis are scattered throughout the chromosome. However, four of these genes are clustered at position 88 (1): *metB*, *metL* and *metF* (coding, respectively, for cystathionine γ-synthase (EC 4.2.99.9)), aspartokinase II-homoserine dehydrogenase II, and 5,10-methylenetetrahydrofolate reductase), and *metJ* which is a pleiotropic regulatory gene coding for a probable aporepressor (2, 3). In order to study the detailed regulation of expression of this gene cluster, we have in a first step cloned the *metJBLF* cluster in plasmid vectors, determined the physical map of the insert, and localized the corresponding genes (4). The clustering of the four *met* genes suggests that an operon may exist for some of these genes and genetic evidence that *metB* and *metL* may belong to the same transcriptional unit has recently been presented (5).

In this paper, we describe the nucleotide sequence of the *metB* gene and of the flanking regions of the *metB* and *metL* genes, the sequence of the *metL* gene having been recently reported by us (6). We also present the characterization of a promoter region upstream from the *metB* gene and the determination of the start site for DNA transcription. The results obtained confirm that *metB* and *metL* form an operon, and that its transcription direction is from *metB* to *metL*. The overall organization of the *met* gene cluster is also analyzed in the light of our results.

MATERIALS AND METHODS

Enzymes and Materials—DNA polymerase large fragment and different restriction endonucleases were obtained from New England Biolabs. T4 polynucleotide kinase was from Boehringer. Acrylamide was from British Drug Houses, Ltd., urea and boric acid were from Serva, hydrazine was from Eastman, and dimethyl sulfate was from Aldrich. All other chemicals, mostly from Merck, were analytical grade or purer. Phenol was distilled.

Cloning of a Promoter Region—The unique *Sma*I site of pKO-1 plasmid (7) was used as a blunt end cloning site. *Alu*I fragments of pMAD4 plasmid (4) were purified on a 4% acrylamide gel. N100 strain ($GalE^-T^+K^-$) was used for transformation experiments (4) and selection was made on MacConkey galactose ampicillin (50 μg/ml) plates.

Bacterial Strains and Growth Media—Media used are described in Miller (8). Strain GT123 is *pyrA53*, *pro-1000*, Δ*thrABC* (9). Strain N100 is *recA*, *galK* (7).

Preparation of Labeled DNA Probes—pMAD4 DNA (4) was restricted with *Pvu*I yielding 4 fragments which were purified on a 6% acrylamide gel. The 2.5-kb[1] fragment carrying the *metB* gene and part of the *metL* gene was restricted with *Mlu*I before 5' end labeling with [γ-^{32}P]ATP (3000 Ci/mmol) and T4 polynucleotide kinase. The two strands of the *MluI-MluI* 408 bp fragment were separated as described by Maxam and Gilbert (10) except that 50% dimethyl sulfoxide was used.

S1 Nuclease Mapping—Mapping of transcription starting point with S1 nuclease was performed as described by Aiba *et al.* (11) RNA (100 μg) extracted from various strains and a few nanograms of a labeled DNA probe (≃50,000 cpm) were mixed, lyophilized, dissolved in 30 μl of buffer (20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 6.5, 80% deionized formamide, 0.4 M NaCl), heated for 10 min at 75 °C in sealed capillaries, and incubated for 3 h at 37 °C. Nonhybridized DNA was digested by adding S1 nuclease, the final volume being 300 μl, and incubated for 1 h at 37 °C. Nucleic acids were extracted with phenol, precipitated with ethanol, and analyzed by electrophoresis on 8% sequencing gels, in parallel with the products of the A + G-sequencing reaction of the DNA probe.

Nucleotide Sequence Determinations—The nucleotide sequences were determined by the chemical method of Maxam and Gilbert (12).

RESULTS AND DISCUSSION

Position of the metB Gene in the Recombinant Plasmid pMAD4—The *metB* gene of *E. coli* was cloned together with the three other *met* genes of the *metJBLF* cluster in plasmid vectors (4). It was localized in the pMAD4 plasmid about 4–5 kb upstream from the single *Eco*RI site. This restriction site has been shown to be near the right (clockwise) end of the approximately 5.6-kb bacterial DNA insert containing the *met* genes (4).

The origin of the *metL* gene is 3835 bp (counterclockwise) from the *Eco*RI site (4, 6). In the present paper, the origin of the *metB* gene was localized precisely by DNA sequencing, at 1163 bp (counterclockwise) from the origin of the *metL* gene. Three ATG codons in phase could theoretically be the translational start codon of the *metB* gene (positions 87, 177, and

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed.

[1] The abbreviations used are: kb, kilobase; bp, base pair.

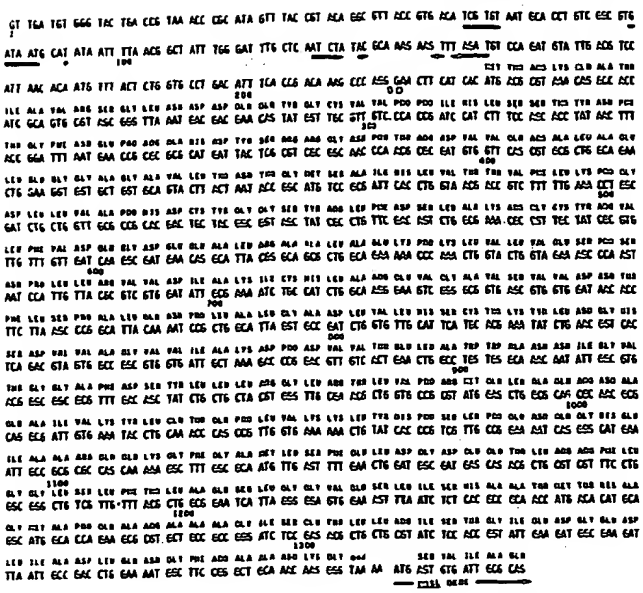


FIG. 1. **Sequence of the *metB* gene and its flanking regions.** The given sequence is that of the noncoding strand. The deduced amino acid sequence for the correct reading frame is shown. The −35 and the −10 regions of the *metBL* promoter are *underlined* with a *thick line*; the *arrows* indicate a palindromic operator-like structure. *SD*: ribosomal attachment sites. * indicates a plausible start translational site for the *metJ* gene (on the complementary strand).

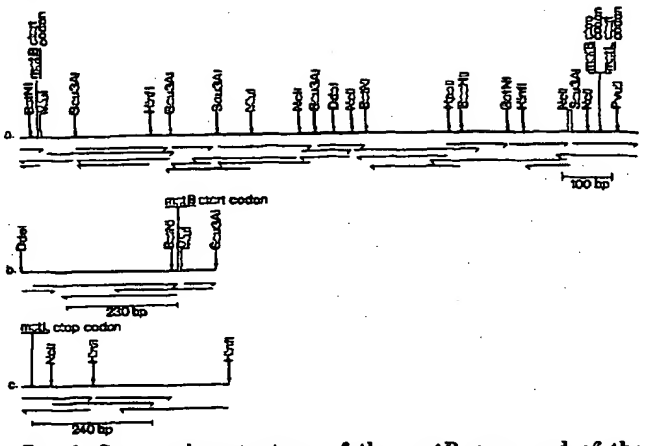


FIG. 2. **Sequencing strategy of the *metB* gene and of the flanking regions of the *metBL* operon.** *a*, restriction map of part of the pBR322-*met* (pMAD4) hybrid plasmid (4) corresponding to the *metB* gene and its sequencing strategy. The *arrows* indicate the sites used for labeling as well as the direction and extent of the sequence. More than 90% of the sequence has been established on both strands. *b*, restriction map of a 230-bp DNA segment situated upstream from the *metB* translational start codon and its sequencing strategy. *c*, restriction map of a 240-bp DNA segment situated downstream from the *metL* gene stop codon (6) and its sequencing strategy.

231 in Fig. 1), but only two of these (positions 177 and 231) are compatible with a gene product having a molecular weight in accordance with the protein size (see "Appendix"[2]). The choice between these two possibilities was made on the basis of the determination of the NH_2-terminal sequence of the enzyme (see "Appendix" and Fig. 1).

DNA Sequence of the metB Gene—The complete nucleotide sequence of the *metB* gene is presented (Fig. 1) as well as the deduced corresponding protein sequence. The DNA fragments and the restriction sites used in the sequence determination are indicated in Fig. 2*a*. The gene is 1,158 nucleotides long and encodes a single polypeptide chain of 386 amino acids. The protein is therefore a tetramer (see "Appendix") of an identical polypeptide chain of M_r = 41,503. Except for minor discrepancies, the deduced overall amino acid composition of the chain is in good agreement with the amino acid composition obtained by analysis of the cystathionine γ-synthase (see "Appendix"). The codon usage in *metB* gene is found to be highly nonrandom. The overall codon usage is similar to that found for example in the *trpA* gene (13), in the *thrA* gene (14), or in the *metL* gene (6).

Localization of a Promoter Region Upstream from the metB Gene—Only 2 bp separate the nonsense codon which terminates the *metB* gene from the translational origin of the *metL* gene (Fig. 1). If *metB* and *metL* constitute a single transcriptional unit (5), a regulatory region for the transcription initiation must be present upstream from the *metB* gene, and a terminator region must be situated downstream from the *metL* gene.

Fig. 1 shows the sequence of 230 nucleotides situated before the start codon of the *metB* gene. The DNA sequence strategy is indicated in Fig. 2*b*. This 230-bp segment is contained within a sequence 700 nucleotides long upstream the origin of the *metB* structural gene. In the corresponding noncoding strand of the *metB* and *metL* genes, this segment of 700 nucleotides does not contain an open reading frame sequence long enough to code for a protein of reasonable length (total sequence not shown).

In order to detect the sequence determining the initiation of *metB* transcription, an *AluI-AluI* fragment of the pMAD4 plasmid was cloned into the pKO-1 vector. This vector carries the selectable *galK* gene which is not expressed if an exogenous promoter sequence is not introduced in the proper position and orientation (7). The *AluI-AluI* fragment is 754 nucleotides long and the sequence of 541 of its nucleotides (up to its 3′ end) is presented in Fig. 1 (positions 1 to 541). It includes part of the *metB* structural gene and 443 nucleotides situated upstream from the start codon of the gene.

The inserted fragment, in both orientations, controls *galK* expression: the recombinant plasmids complement a $GalE^+T^+K^-$ host. The orientations of the inserts in the recombinant plasmids were confirmed by restriction mapping and DNA sequencing. From these results, we can conclude that the 754-bp *AluI-AluI* fragment has the *metB* promoter activity in one orientation, and another promoter activity in the other orientation (see below).

We decided to localize the *metB* promoter on a shorter fragment. Digestion with *RsaI* of the *AluI-AluI* fragment and cloning of the resulting mixture into the pKO-1 vector gave the following result: a 430-nucleotide *RsaI-RsaI* fragment (still containing a *RsaI* site) (positions 13–442 from Fig. 1) still has a promoter activity. The structure of this inserted fragment was confirmed by DNA sequencing.

Determination of the Start Site for metB Transcription by S1 Nuclease Mapping—Nuclease S1 mapping was used to map the location of the 5′ ends of RNA molecules in relation to specific sites within the template DNA. Total RNA was extracted from cells (GT123) harboring a multicopy plasmid (pMAD4) which carries the *metJBLF* gene cluster, or from cells without any plasmid. The *metB*-specific mRNA was then allowed to hybridize with a single-stranded DNA probe labeled in 5′ at its *MluI* restricted end. This probe was obtained from a *MluI-MluI* fragment 408 nucleotides long, the 3′ sequence of which is presented in Fig. 1 (positions 1 to 238).

[2] The "Appendix" is found on pp. 14872–14873.

The hybrids were then digested with S1 nuclease and analyzed by gel electrophoresis in parallel with fragments obtained by chemical cleavage of the original probe (Fig. 4). We conclude that the 3′ ends of the undigested DNA fragments are located at 139 to 141 nucleotides from the *Mlu*I-labeled end in the coding DNA strand (positions 99, 98, and 97, respectively, in

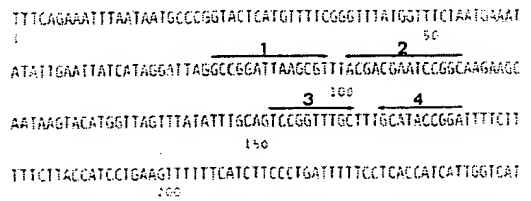


FIG. 3. **Sequence of a 240-bp DNA segment situated downstream from the *metL* gene.** The first nucleotide in the figure follows the *metL* gene stop codon. *Arrows* (*1* and 2) indicate regions homologous to those described by Higgins *et al.* (17) (see text and Fig. 5*a*). *Arrows 3* and *4* indicate regions of dyad symmetry relevant to the possible *rho*-independent termination signal (see text and Fig. 5*b*).

FIG. 4. **Identification of the start point for the *metBL* operon transcription by S1 nuclease mapping.** A 408-bp *Mlu*I-*Mlu*I single-stranded DNA probe (labeled in 5′) containing the *metB* control region was hybridized with RNA extracted from a strain containing the *metJBLF* plasmid (pMAD4) (grown in M63 glucose supplemented with uracil, tetracycline, casamino acids, and diaminopimelic acid) and treated with S1 nuclease. The protected fragments (*Lane 1*) were loaded in parallel with the products of the A + G reactions of the original probe (*Lane 2*) on a 8% acrylamide-8 M urea sequencing gel.

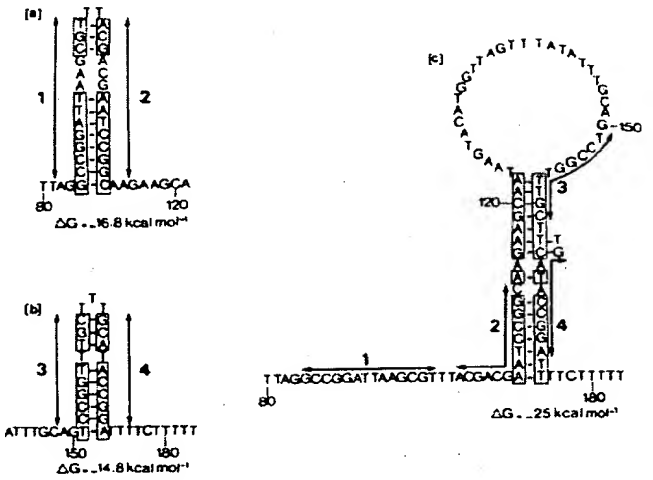


FIG. 5. **Proposed terminator region of the *metBL* operon.** Three possible stem and loop structures are shown. The free energies of the structures were calculated according to Tinoco *et al.* (22).

Fig. 1). As previously described in other cases (11), the S1 nuclease digestion products showed minor length heterogeneity. No mRNA specific of *metB* was detected when the plasmid was absent (data not shown); the mRNA molecules transcribed from the chromosomal copy of *metB* could not be detected since the cells were grown under repressing conditions. The start point of transcription determined here (positions 99, 98, and 97 in Fig. 1) allows us to identify the Pribnow box and the −35 region of the *metB* promoter (*underlined* in Fig. 1).

To complete the analysis of the DNA region situated upstream from the *metB* structural gene in Fig. 1, a possible ribosome attachment site (15) is indicated, 10 bp before the translational start point of the *metB* gene, as well as a region possessing dyad symmetry which could be an operator-like structure.

Putative Terminator Region Situated Downstream from the metL gene—Fig. 3 shows the nucleotide sequence of 240 bp downstream from the stop codon of the *metL* gene (6). The DNA sequence strategy is indicated in Fig. 2*c*. A careful analysis of this sequence reveals the following features. (*a*) It does not contain an in-phase sequence long enough to code for a protein. (*b*) At positions 151 to 183 a typical *rho*-independent terminator structure (16) is found ($\Delta G = -14.8$ kcal·mol^{-1}) (Fig. 5*b*), possibly responsible for a termination signal of transcription. (*c*) Before this sequence, between positions 80 to 121, a potential stem and loop structure ($\Delta G = -16.9$ kcal·mol^{-1}) can be formed (Fig. 5*a*). The nucleotide sequence of this structure is very similar to the consensus structure described for several intercistronic regions (17). Recently, this DNA palindromic segment has been reported to occur also between operons.[3] The function of these structures has not been elucidated yet, but it has been postulated that they can be involved in the regulation of transcription termination events.[2] Moreover, a more stable single stem and loop structure can be formed, encompassing the two structures already described ($\Delta G = -25$ kcal·mol^{-1}) (Fig. 5*c*).

That a terminator region must lie within this 250-bp segment is further substantiated by the following argument. The *metF* gene with its own promoter (18) is situated at the right (clockwise) end of the bacterial DNA insert, near the single *Eco*RI site of the plasmid (see above) (4). This restriction site

[3] J. M. Clément and M. Hofnung, personal communication.

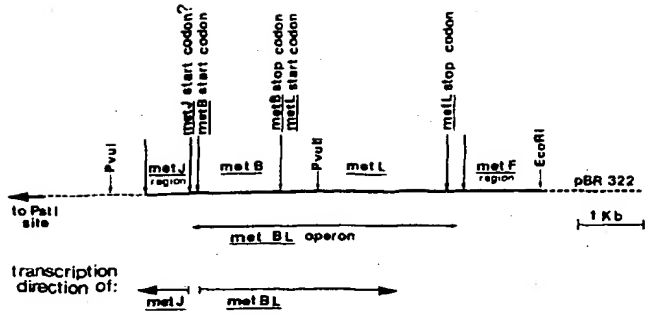


FIG. 6. **Overall organization of the *metJBLF* gene cluster in the pBR322-*met*(pMAD4) hybrid plasmid (4).** The sequenced regions presented are: the entire *metB* gene, 230 bp upstream from the *metB* start codon, and 240 bp downstream from the *metL* stop codon. The nucleotide sequence of the *metL* gene (6) and of the *metF* gene (19) were previously published and that of *metJ* is to be published.

is 1402 bp from the stop codon of the *metL* gene. This leaves 1162 bp (1402 bp minus 240 bp of the putative terminator region) for the *metF* structural gene and its flanking regions. This figure agrees with the actual size of the *metF* structural gene and its regulatory sequences (19).

A recapitulation of the results presented in the last three sections indicates that, in the noncoding strand depicted in Figs. 1 and 3 no open reading frame exists in the DNA segments situated immediately upstream from the *metB* gene or downstream from the *metL* gene. Also, a promoter region has been localized upstream from the *metB* gene and a possible terminator region can be identified downstream from the *metL* gene according to the consensus structures previously described (16). This, together with the fact that only 2 bp separate the *metB* and *metL* genes, confirm the genetic evidence that *metB* and *metL* form an operon.

Overall Organization of the metJBLF Cluster—Since the transducing phage (20, 21) from which pMAD4 was constructed (4) contains *metJ* in addition to the other genes of the *met* cluster, we may ask the question of its presence in the plasmid. If the answer is positive, where is it located with respect to *metB*, *metL*, and *metF* which are now well positioned?

Complementation experiments between a *metJ* strain (Gif 881L, resistant to norleucine) and the pMAD4 plasmid have shown us that *metJ* is actually present in the plasmid. The total *metJBLF* cluster occupies about 5.6 kb (4); since the *metF* region is situated at the right (clockwise) end of the bacterial DNA insert in pMAD4 and the total size of the *metBL* and *metF* regions is of 5.1 kb, *metJ* must lie within the 700-bp DNA segment situated upstream from the origin of the *metB* gene. However, no open reading frame exists in one of the strands (the non-coding strand for *metBL*) of this segment (see above); consequently, the only possibility is that *metJ* is transcribed on the opposite strand. Actually, in this complementary strand, only one open reading frame coding for a protein of 151 residues is found, starting 139 bp before the translational start codon of *metB* (Fig. 1). Moreover, an *AluI-AluI* fragment (754 nucleotides) has been demonstrated to have a promoter activity when inserted in the pKO-1 vector in both orientations (see above). We postulate that this DNA fragment has the *metBL* promoter activity in one orientation and the *metJ* promoter activity in the other orientation. However, further experiments are needed to confirm the exact translational start site of the *metJ* gene.

All the information collected allows us to propose a more precise overall organization of the *metJBLF* cluster of *E. coli*. The size of the cluster is about 5.7 kb, with *metB* and *metL* forming an operon of 4 kb, leaving *metF* to its right (clockwise) in a 1.1-kb region and *metJ* to its left (counterclockwise) in a 0.6-kb region (Fig. 6).

Finally, no evidence for an attenuation mechanism with a region specifying a leader peptide and a terminator structure was found for the *metBL* operon. This in conjunction with the existence of a pleiotropic dominant regulatory gene (*metJ*) (2, 3) points to a negative control mechanism and to the presence of an operator region for this operon. The palindromic sequence *underlined* in Fig. 1 is compatible with this possibility.

Acknowledgments—We are grateful to R. C. Greene for helpful discussions, M. Debarbouillé and C. Chapon for detailed discussions about the S1 mapping methodology, F. Rougeon for the generous gift of S1 nuclease, and M. Rosenberg for the generous gift of the pKO-1 plasmid and the N100 strain. We would like to thank D. Margarita for excellent technical assistance in the promoter cloning experiments, and L. Girardot for typing the manuscript.

REFERENCES

1. Bachmann, B. J., and Low, K. B. (1980) *Bacteriol. Rev.* **44**, 1–56
2. Patte, J.-C., Le Bras, G., and Cohen, G. N. (1967) *Biochim. Biophys. Acta* **136**, 245–257
3. Su, C. H., and Greene, R. C. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 367–371
4. Zakin, M. M., Greene, R. C, Dautry-Varsat, A., Duchange, N., Ferrara, P., Py, M.-C., Margarita, D., and Cohen, G. N. (1982) *Mol. Gen. Genet.* **187**, 101–106
5. Greene, R. C., and Smith, A. A. (1982) *Fed. Proc.* **41**, 752
6. Zakin, M. M, Duchange, N., Ferrara, P., and Cohen, G. N. (1983) *J. Biol. Chem.* **258**, 3028–3031
7. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C., and Rosenberg, M. (1981) in *Gene Amplification and Analysis* (Chirikjian, J., and Papas, T., eds) Vol. 2, pp. 383–415, Elsevier, New York
8. Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9. Cossart, P., Katinka, M., Yaniv, M., Saint Girons, I., and Cohen, G. N. (1979) *Mol. Gen. Genet.* **175**, 39–44
10. Maxam, A. M., and Gilbert, W. (1980) *Methods Enzymol.* **65**, 525–526
11. Aiba, H., Adhya, S., and de Combrugghe, B. (1981) *J. Biol. Chem.* **256**, 11905–11910
12. Maxam, A. M., and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 560–564
13. Nichols, B. P., and Yanofsky, C. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 5244–5248
14. Katinka, M., Cossart, P., Sibilli, L., Saint-Girons, I., Chalvignac, M. A., Le Bras, G., Cohen, G. N., and Yaniv, M. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 5730–5733
15. Shine, J., and Dalgarno, L. (1975) *Nature (Lond.)* **254**, 34–38
16. Rosenberg, M., and Court, D. (1979) *Annu. Rev. Genet.* **13**, 319–353
17. Higgins, C. F., Ames, G. F.-L., Barnes, W. M., Clement, J. M., and Hofnung, M. (1982) *Nature (Lond.)* **298**, 760–762
18. Greene, R. C., Krueger, J. H., and Johnson, J. R. (1982) *Mol. Gen. Genet.* **187**, 401–404
19. Saint-Girons, I., Duchange, N., Zakin, M. M., Lark, I., Margarita, D., Ferrara, L., and Cohen, G. N. (1983) *Nucleic Acids Res.* **11**, 6723–6732
20. Press, R., Glansdorff, N., Miner, P., de Vries, J., Kadner, R., and Maas, W. K. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 795–798
21. Ohtsubo, E., Lee, H. J., Deonier, R. C., and Davidson, N. (1974) *J. Mol. Biol.* **89**, 599–618
22. Tinoco, I., Jr., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M., and Gralla, J. (1973) *Nature New Biol.* **246**, 40–41

Additional references are found on p. 14873.

RELATED PROCEEDINGS APPENDIX

None.

Intellectual Property Library ISSN 1526-8535

FULL TEXT OF CASES (USPQ2D)
Cases Publishing the Week of Oct 10, 2005

Capon v. Eshhar, 76 USPQ2d 1078 (CA FC 2005)

76 USPQ2D 1078
Capon v. Eshhar
U.S. Court of Appeals Federal Circuit

Nos. 03-1480, -1481
Decided August 12, 2005

Headnotes

PATENTS

[1] Patentability/Validity — Specification — Written description (§115.1103)

Board of Patent Appeals and Interferences erred in holding that interference parties' specifications for claims directed to chimeric genes designed to enhance immune response do not satisfy written description requirement of 35 U.S.C. §112, even though specifications do not include complete nucleotide sequences of claimed genes, since written description requirement must be applied in context of particular invention and state of knowledge, and there is no per se rule that nucleotide sequence must be recited anew when that information is already known in art, since invention at issue lies not in discovering which DNA segments are related to immune response, but in novel combination of segments to achieve novel result, since claimed chimeric genes are prepared from known DNA sequences of known function, and since requirement that these sequences be analyzed and reported in specifications therefore does not add descriptive substance.

[2] Patentability/Validity — Specification — Written description (§115.1103)

Patentability/Validity — Specification — Enablement (§115.1105)

Patent construction — Claims — Broad or narrow (§125.1303)

Determination of adequate support for generic claims to biological subject matter depends, among other factors, on existing knowledge in field, extent and content of prior art, maturity of science or technology, predictability of aspect at issue, and other considerations appropriate to claimed subject matter; in present case, Board of Patent Appeals and Interferences erred in failing to address support for each of parties' claims directed to chimeric genes designed to enhance immune response, since parties presented general teachings as to how to select and recombine DNA, as well as specific examples of production of specified chimeric genes, since they used standard systems of description and identification, as well as known procedures for selecting, isolating, and linking known DNA segments, since board's repeated observation that full scope of claims appears to be "enabled" cannot be reconciled with its objection that only "general plan" to combine unidentified DNA is presented, since whether inventors demonstrated sufficient generality to support scope of claims must be determined claim by claim, and since board's position that inventions at issue were merely "invitation to experiment"did not distinguish among broad and narrow claims, and concerns enablement more than written description.

Particular Patents

Particular patents — Chemical — Chimeric genes

6,407,221, Capon, Weiss, Irving, Roberts, and Zsebo, chimeric chains for receptor-associated signal transduction pathways, cancellation of claims corresponding to count in interference no. 103,887, for failure to satisfy written description requirement, vacated and remanded.

Case History and Disposition

Appeal from the U.S. Patent and Trademark Office, Board of Patent Appeals and Interferences.

Patent interference proceeding (no. 103,887) between Daniel J. Capon,

Arthur Weiss, Brian A. Irving, Margo R. Roberts, and Krisztina Zsebo (patent no. 6,407,221), and Zelig Eshhar, Daniel Schindler, Tova Waks, and Gideon Gross (application serial no. 08/084,994). Both parties appeal from cancellation of claims corresponding to interference count on ground that neither party met written description requirement. Jon Dudas, in his capacity as Director of the PTO, intervenes in support of the board. Vacated and remanded.

Attorneys:

Steven B. Kelber, of Piper Rudnick, Washington, D.C., for appellants.

Roger L. Browdy, of Browdy and Neimark, Washington, for cross-appellants.

Mary L. Kelly, associate solicitor, John M. Whealan, solicitor, and Stephen Walsh, associate solicitor, U.S. Patent and Trademark Office, Arlington, Va., for intervenor.

Judge:

Before Newman, Mayer,* and Gajarsa, circuit judges.

Footnotes

* Haldane Robert Mayer vacated the position of Chief Judge on December 24, 2004.

Opinion Text

Opinion By:

Newman, J.

Both of the parties to a patent interference proceeding have appealed the decision of the Board of Patent Appeals and Interferences of the United States Patent and Trademark Office, wherein the Board held that the specification of neither party met the written description requirement of the patent statute. *Capon v. Eshhar*, Interf. No. 103,887 (Bd. Pat. App. & Interf. Mar. 26, 2003). The Board dissolved the interference and cancelled all of the claims of both parties corresponding to the interference count. With this ruling, the Board terminated the proceeding and did not reach the question of priority of invention. We conclude that the Board erred in its application of the law of written description. The decision is vacated and the case is remanded to the Board for further proceedings.

BACKGROUND

Daniel J. Capon, Arthur Weiss, Brian A. Irving, Margo R. Roberts, and Krisztina Zsebo (collectively "Capon") and Zelig Eshhar, Daniel Schindler, Tova Waks, and Gideon Gross (collectively "Eshhar") were the parties to an interference proceeding between Capon's United States Patent No. 6,407,221 ("the '221 patent") entitled "Chimeric Chains for Receptor-Associated Signal Transduction Pathways" and Eshhar's patent application Serial No. 08/084,994 ("the '994 application") entitled "Chimeric Receptor Genes and Cells Transformed Therewith."Capon's Patent No. 5,359,046 ("the '046 patent"), parent of the '221 patent, was also included in the interference but was held expired for non-payment of a maintenance fee. The PTO included the '046 patent in its decision and in its argument of this appeal.1

A patent interference is an administrative proceeding pursuant to 35 U.S.C. §§102(g) and 135(a), conducted for the purpose of determining which of competing applicants is the first inventor of common subject matter. An interference is instituted after the separate patent applications have been examined and found to contain patentable subject matter. Capon's patents had been examined and had issued before this interference was instituted, and Eshhar's application had been examined and allowed but a patent had not yet issued.

During an interference proceeding the Board is authorized to determine not only priority of invention but also to redetermine patentability. 35 U.S.C. §6(b). The question of patentability of the claims of both parties was raised *sua sponte* by an administrative patent judge during the preliminary proceedings. Thereafter the Board conducted an *inter partes* proceeding limited to this question, receiving evidence and argument. The Board then invalidated all of the claims that had been designated as corresponding to the count of the interference, *viz.*, all of the claims of the Capon '221 patent, claims 5-8 of the Capon '046 patent, and claims 1-7, 9-20, and 23 of the Eshhar '994 application.

In accordance with the Administrative Procedure Act, the law as interpreted and applied by the agency receives plenary review on appeal, and the agency's factual findings are reviewed to determine whether they were arbitrary, capricious, or unsupported by substantial evidence in the administrative record. *See* 5 U.S.C. §706(2); *Dickinson v. Zurko*, 527 U.S. 150, 164-65 [50 USPQ2d 1930] (1999); *In re Gartside*, 203 F.3d 1305, 1315 [53 USPQ2d 1769] (Fed. Cir. 2000).

The Invention

A chimeric gene is an artificial gene that combines segments of DNA in a way that does not occur in nature. The '221 patent and '994 application are directed to the production of chimeric genes designed to enhance the immune response by providing cells with specific cell-surface antibodies in a form that can penetrate diseased sites, such as solid tumors, that were not previously reachable. The parties explain that their invention is a way of endowing immune cells with antibody-type specificity, by combining known antigen-binding-domain producing DNA and known lymphocyte-receptor-protein producing DNA into a unitary gene that can express a unitary polypeptide chain. Eshhar summarized the problem to which the invention is directed:

Antigen-specific effector lymphocytes, such as tumor-specific T cells, are very rare,

individual-specific, limited in their recognition spectrum and difficult to obtain against most malignancies. Antibodies, on the other hand, are readily obtainable, more easily derived, have wider spectrum and are not individual-specific. The major problem of applying specific antibodies for cancer immunotherapy lies in the inability of sufficient amounts of monoclonal antibodies (mAb) to reach large areas within solid tumors. Technical Paper Explaining Eshhar's Invention, at 6.

The inventions of Capon and Eshhar are the chimeric DNA that encodes single-chain chimeric proteins for expression on the surface of cells of the immune system, plus expression vectors and cells transformed by the chimeric DNA. The experts for both parties explain that the invention combines selected DNA segments that are both endogenous and nonendogenous to a cell of the immune system, whereby the nonendogenous segment encodes the single-chain variable ("scFv") domain of an antibody, and the endogenous segment encodes cytoplasmic, transmembrane, and extracellular domains of a lymphocyte signaling protein. They explain that the scFv domain combines the heavy and light variable ("Fv") domains of a natural antibody, and thus has the same specificity as a natural antibody. Linking this single chain domain to a lymphocyte signaling protein creates a chimeric scFv-receptor ("scFvR") gene which, upon transfection into a cell of the immune system, combines the specificity of an antibody with the tissue penetration, cytokine production, and target-cell destruction capability of a lymphocyte.

The parties point to the therapeutic potential if tumors can be infiltrated with specifically designed immune cells of appropriate anti-tumor specificity.

The Eshhar Claims

The Board held unpatentable the following claims of Eshhar's '994 application; these were all of the '994 claims that had been designated as corresponding to the count of the interference. Eshhar's claim 1 was the designated count.

1. A chimeric gene comprising

a first gene segment encoding a single-chain Fv domain (scFv) of a specific antibody and

a second gene segment encoding partially or entirely the transmembrane and cytoplasmic, and optionally the extracellular, domains of an endogenous protein

wherein said endogenous protein is expressed on the surface of cells of the immune system and triggers activation and/or proliferation of said cells,

which chimeric gene, upon transfection to said cells of the immune system, expresses said scFv domain and said domains of said endogenous protein in one single chain on the surface of the transfected cells such that the transfected cells are triggered to activate and/or proliferate and have MHC nonrestricted antibody-type specificity when said expressed scFV domain binds to its antigen.

2. A chimeric gene according to claim 1 wherein the second gene segment further

comprises partially or entirely the extracellular domain of said endogenous protein.
3. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against tumor cells.
4. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against virus infected cells.
5. A chimeric gene according to claim 4 wherein the virus is HIV.
6. A chimeric gene according to claim 1 wherein the second gene segment encodes a lymphocyte receptor chain.
7. A chimeric gene according to claim 6 wherein the second gene segment encodes a chain of the T cell receptor.
9. A chimeric gene according to claim 7 wherein the second gene segment encodes the a, b, g, or d chain of the antigen-specific T cell receptor.
10. A chimeric gene according to claim 1 wherein the second gene segment encodes a polypeptide of the TCR/CD3 complex.
11. A chimeric gene according to claim 10 wherein the second gene segment encodes the zeta or eta isoform chain.
12. A chimeric gene according to claim 1 wherein the second gene segment encodes a subunit of the Fc receptor or IL-2 receptor.

13. A chimeric gene according to claim 12 wherein the second gene segment encodes a common subunit of IgE and IgG binding Fc receptors.
14. A chimeric gene according to claim 13 wherein said subunit is the gamma subunit.
15. A chimeric gene according to claim 13 wherein the second gene segment encodes the CD16a chain of the FcgRIII or FcgRII.
16. A chimeric gene according to claim 12 wherein the second gene segment encodes the a or b subunit of the IL-2 receptor.
17. An expression vector comprising a chimeric gene according to claim 1.
18. A cell of the immune system endowed with antibody specificity transformed with an expression vector according to claim 17.
19. A cell of the immune system endowed with antibody specificity comprising a chimeric gene according to claim 1.
20. A cell if the immune system according to claim 19 selected from the group consisting of a natural killer cell, a lymphokine activated killer cell, a cytotoxic T cell, a helper T cell and a subtype thereof.
23. A chimeric gene according to claim 1 wherein said endogenous protein is a lymphocyte receptor chain, a polypeptide of the TCR/CD3 complex, or a subunit of the Fc or IL-2 receptor.

The Board did not discuss the claims separately, and held that the specification failed to satisfy the written description requirement as to all of these claims.

The Capon Claims

Claims 1-10, all of the claims of the '221 patent, were held unpatentable on written description grounds. Claims 1-6 are directed to the chimeric DNA, claims 7, 8, and 10 to

the corresponding cell comprising the DNA, and claim 9 to the chimeric protein:

1. A chimeric DNA encoding a membrane bound protein, said chimeric DNA comprising in reading frame:

DNA encoding a signal sequence which directs said membrane bound protein to the surface membrane;

DNA encoding a non-MHC restricted extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least one ligand is a protein on the surface of a cell or a viral protein;

DNA encoding a transmembrane domain which is obtained from a protein selected from the group consisting of CD4, CD8, immunoglobulin, the CD3 zeta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain; and

DNA encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system which is obtained from CD3 zeta,

wherein said extracellular domain and said cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric DNA is expressed as a membrane bound protein in a host cell under conditions suitable for expression, said membrane bound protein initiates signaling in said host cell when said extracellular domain binds said at least one ligand.

2. The DNA of claim 1, wherein said single-chain antibody recognizes an antigen selected from the group consisting of viral antigens and tumor cell associated antigens.

3. The DNA of claim 2 wherein said single-chain antibody is specific for the HIV env glycoprotein.

4. The DNA of claim 1, wherein said transmembrane domain is naturally joined to said cytoplasmic domain.

5. An expression cassette comprising a transcriptional initiation region, the DNA of claim 1 under the transcriptional control of said transcriptional initiation region, and a transcriptional termination region.

6. A retroviral RNA or DNA construct comprising the expression cassette of claim 5.

7. A cell comprising the DNA of claim 1.

8. The cell of claim 7, wherein said cell is a human cell.

9. A chimeric protein comprising in the N-terminal to C-terminal direction:

a non-MHC restricted extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least

one ligand is a protein on the surface of a cell or a viral protein;

a transmembrane domain which is obtained from a protein selected from the group consisting CD4, CD8, immunoglobulin, the CD3 zeta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain; and

a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system which is obtained from CD3 zeta,

wherein said extracellular domain and said cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined to an extracellular

> ligand-binding domain, and when said chimeric protein is expressed as a membrane bound protein in a host cell under conditions suitable for expression, said membrane bound protein initiates signaling in said host cell when said extracellular domain binds said at least one ligand.
>
> 10. A mammalian cell comprising as a surface membrane protein, the protein of claim 9.

In addition, claims 5, 6, 7, and 8 of Capon's '046 patent were held unpatentable. These claims are directed to chimeric DNA sequences where the encoded extracellular domain is a single-chain antibody containing ligand binding activity.

The Board Decision

The Board presumed enablement by the specifications of the '221 patent and '994 application of the full scope of their claims, and based its decision solely on the ground of failure of written description. The Board held that neither party's specification provides the requisite description of the full scope of the chimeric DNA or encoded proteins, by reference to knowledge in the art of the "structure, formula, chemical name, or physical properties"of the DNA or the proteins. In the Board's words:

> We are led by controlling precedent to understand that the full scope of novel chimeric DNA the parties claim is not described in their specifications under 35 U.S.C. §112, first paragraph, by reference to contemporary and/or prior knowledge in the art of the structure, formula, chemical name, or physical properties of many protein domains, and/or DNA sequences which encode many protein domains, which comprise single-chain proteins and/or DNA constructs made in accordance with the plans, schemes, and examples thereof the parties disclose.

Bd. op. at 4. As controlling precedent the Board cited *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559 [43 USPQ2d 1398] (Fed. Cir. 1997); *Fiers v. Revel Co.*, 984 F.2d 1164 [25 USPQ2d 1601] (Fed. Cir. 1993); *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200 [18 USPQ2d 1016] (Fed. Cir. 1991); and *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 296 F.3d 1316 [63 USPQ2d 1609] (Fed. Cir. 2002). The Board summarized its holding as follows:

> Here, both Eshhar and Capon claim novel genetic material described in terms of the functional characteristics of the protein it encodes. Their specifications do not satisfy the written description requirement because persons having ordinary skill in the art would not have been able to visualize and recognize the identity of the claimed genetic material without considering additional knowledge in the art, performing additional experimentation, and testing to confirm results.

Bd. op. at 89.

DISCUSSION

Eshhar and Capon challenge both the Board's interpretation of precedent and the Board's ruling that their descriptions are inadequate. Both parties explain that their chimeric genes are produced by selecting and combining known heavy- and light-chain immune-related DNA segments, using known DNA-linking procedures. The specifications of both parties describe procedures for identifying and obtaining the desired immune-related DNA segments and linking them into the desired chimeric genes. Both

parties point to their specific examples of chimeric DNA prepared using identified known procedures, along with citation to the scientific literature as to every step of the preparative method.

The parties presented expert witnesses who placed the invention in the context of prior knowledge and explained how the descriptive text would be understood by persons of skill in the field of the invention. The witnesses explained

that the principle of forming chimeric genes from selected segments of DNA was known, as well as their methods of identifying, selecting, and combining the desired segments of DNA. Dr. Eshhar presented an expert statement wherein he explained that the prior art contains extensive knowledge of the nucleotide structure of the various immune-related segments of DNA; he stated that over 785 mouse antibody DNA light chains and 1,327 mouse antibody DNA heavy chains were known and published as early as 1991. Similarly Capon's expert Dr. Desiderio discussed the prior art, also citing scientific literature:

> The linker sequences disclosed in the '221 patent (col. 24, lines 4 and 43) used to artificially join a heavy and light chain nucleic acid sequence and permit functional association of the two ligand binding regions were published by 1990, as were the methods for obtaining the mature sequences of the desired heavy and light chains for constructing a SAb (Exhibit 47, Batra et al., J., Biol. Chem., 1990; Exhibit 48, Bird et al., Science, 1988; Exhibit 50, Huston et al., PNAS, 1988; Exhibit 51, Chaudhary, PNAS, 1990, Exhibit 56, Morrison et al., Science, 1985; Exhibit 53, Sharon et al., Nature 1984).

Desiderio declaration at 4 ¶ 11.

Both parties stated that persons experienced in this field would readily know the structure of a chimeric gene made of a first segment of DNA encoding the single-chain variable region of an antibody, and a second segment of DNA encoding an endogenous protein. They testified that re-analysis to confirm these structures would not be needed in order to know the DNA structure of the chimeric gene, and that the Board's requirement that the specification must reproduce the "structure, formula, chemical name, or physical properties" of these DNA combinations had been overtaken by the state of the science. They stated that where the structure and properties of the DNA components were known, reanalysis was not required.

Eshhar's specification contains the nucleotide sequences of sixteen different receptor primers and four different scFv primers from which chimeric genes encoding scFvR may be obtained, while Capon's specification cites literature sources of such information. Eshhar's specification shows the production of chimeric genes encoding scFvR using primers, as listed in Eshhar's Table I. Capon stated that natural genes are isolated and joined using conventional methods, such as the polymerase chain reaction or cloning by primer repair. Capon, like Eshhar, discussed various known procedures for identifying, obtaining, and linking DNA segments, accompanied by experimental examples. The Board did not dispute that persons in this field of science could determine the structure or formula of the linked DNA from the known structure or formula of the components.

The Board stated that "controlling precedent" required inclusion in the specification of the complete nucleotide sequence of "at least one"chimeric gene. Bd. op. at 4. The Board also objected that the claims were broader than the specific examples. Eshhar and Capon each responds by pointing to the scientific completeness and depth of their descriptive texts, as well as to their illustrative examples. The Board did not relate any of the claims, broad or narrow, to the examples, but invalidated all of the claims without analysis of their scope and the relation of claim scope to the details of the specifications.

Eshhar and Capon both argue that they have set forth an invention whose scope is fully and fairly described, for the nucleotide sequences of the DNA in chimeric combination is readily understood to contain the nucleotide sequences of the DNA components. Eshhar points to the general and specific description in his specification of known immune-related DNA segments, including the examples of their linking. Capon points similarly to his description of selecting DNA segments that are known to express immune-related proteins, and stresses the existing knowledge of these segments and their nucleotide sequences, as well as the known procedures for selecting and combining DNA segments, as cited in the specification.

Both parties argue that the Board misconstrued precedent, and that precedent does not establish a *per se* rule requiring nucleotide-by-nucleotide re-analysis when the structure of the component DNA segments is already known, or readily determined by known procedures.

The Statutory Requirement

The required content of the patent specification is set forth in Section 112 of Title 35:

> §112 ¶ 1. The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The "written description"requirement implements the principle that a patent must describe the technology that is sought to be patented; the requirement serves both to satisfy the inventor's obligation to disclose the technologic knowledge upon which the patent is based, and to demonstrate that the patentee was in possession of the invention that is claimed. *See Enzo Biochem*, 296 F.3d at 1330 (the written description requirement "is the quid pro quo of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time"); *Reiffin v. Microsoft Corp.*, 214 F.3d 1342, 1345-46 [54 USPQ2d 1915] (Fed. Cir. 2000) (the purpose of the written description requirement "is to ensure that the scope of the right to exclude ... does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification"); *In re Barker*, 559 F.2d 588, 592 n.4 [194 USPQ 470] (CCPA 1977) (the goal of the written description requirement is "to clearly convey the information that

an applicant has invented the subject matter which is claimed"). The written description requirement thus satisfies the policy premises of the law, whereby the inventor's technical/scientific advance is added to the body of knowledge, as consideration for the grant of patent exclusivity.

The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science.

For the chimeric genes of the Capon and Eshhar inventions, the law must take cognizance of the scientific facts. The Board erred in refusing to consider the state of the scientific knowledge, as explained by both parties, and in declining to consider the separate scope of each of the claims. None of the cases to which the Board attributes the requirement of total DNA re-analysis, *i.e.*, *Regents v. Lilly, Fiers v. Revel, Amgen*, or *Enzo Biochem*, require a re-description of what was already known. In *Lilly*, 119 F.3d at 1567, the cDNA for human insulin had never been characterized. Similarly in *Fiers*, 984 F.2d at 1171, much of the DNA sought to be claimed was of unknown structure, whereby this court viewed the breadth of the claims as embracing a "wish"or research "plan." In *Amgen*, 927 F.2d at 1206, the court explained that a novel gene was not adequately characterized by its biological function alone because such a description would represent a mere "wish to know the identity" of the novel material. In *Enzo Biochem*, 296 F.3d at 1326, this court reaffirmed that deposit of a physical sample may replace words when description is beyond present scientific capability. In *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1332 [65 USPQ2d 1385] (Fed. Cir. 2003) the court explained further that the written description requirement may be satisfied "if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." These evolving principles were applied in *Noelle v. Lederman*, 355 F.3d 1343, 1349 [69 USPQ2d 1508] (Fed. Cir. 2004), where the court affirmed that the human antibody there at issue was not adequately described by the structure and function of the mouse antigen; and in *University of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 925-26 [69 USPQ2d 1886] (Fed. Cir. 2004), where the court affirmed that the description of the COX-2 enzyme did not serve to describe unknown compounds capable of selectively inhibiting the enzyme.

[1] The "written description" requirement must be applied in the context of the particular invention and the state of the knowledge. The Board's rule that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known, is an inappropriate generalization. When the prior art includes the nucleotide information, precedent does not set

a *per se* rule that the information must be determined afresh. Both parties state that a person experienced in the field of this invention would know that these known DNA segments would retain their DNA sequences when linked by known methods. Both parties explain that their invention is not in discovering which DNA segments are related to the immune response, for that is in the prior art, but in the novel combination of the DNA segments to achieve a novel result.

The "written description" requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution. Both Eshhar and Capon explain that this invention does not concern the discovery of gene function or structure, as in *Lilly*. The chimeric genes here at issue are prepared from known DNA sequences of known function. The Board's requirement that these sequences must be analyzed and reported in the specification does not add descriptive substance. The Board erred in holding that the specifications do not meet the written description requirement because they do not reiterate the structure or formula or chemical name for the nucleotide sequences of the claimed chimeric genes.

Claim Scope

There remains the question of whether the specifications adequately support the breadth of all of the claims that are presented. The Director argues that it cannot be known whether all of the permutations and combinations covered by the claims will be effective for the intended purpose, and that the claims are too broad because they may include inoperative species. The inventors say that they have provided an adequate description and exemplification of their invention as would be understood by persons in the field of the invention. They state that biological properties typically vary, and that their specifications provide for evaluation of the effectiveness of their chimeric combinations.

It is well recognized that in the "unpredictable" fields of science, it is appropriate to recognize the variability in the science in determining the scope of the coverage to which the inventor is entitled. Such a decision usually focuses on the exemplification in the specification. *See, e.g., Enzo Biochem*, 296 F.3d at 1327-28 (remanding for district court to determine "[w]hether the disclosure provided by the three deposits in this case, coupled with the skill of the art, describes the genera of claims 1-3 and 5"); *Lilly*, 119 F.3d at 1569 (genus not described where "a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus" had not been provided); *In re Gostelli*, 872 F.2d 1008, 1012 [10 USPQ2d 1614] (Fed. Cir. 1989) (two chemical compounds were insufficient description of subgenus); *In re Smith*, 458 F.2d 1389, 1394-95 [173 USPQ 679] (CCPA 1972) (disclosure of genus and one species was not sufficient description of intermediate subgenus); *In re Grimme*, 274 F.2d 949, 952 [124 USPQ 499] (CCPA 1960) (disclosure of single example and statement of scope sufficient disclosure of subgenus).

[2] Precedent illustrates that the determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or

technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter. *See, e.g., In re Wallach*, 378 F.3d 1330, 1333-34 [71 USPQ2d 1939] (Fed. Cir. 2004) (an amino acid sequence supports "the entire genus of DNA sequences"that can encode the amino acid sequence because "the state of the art has developed" such that it is a routine matter to convert one to the other); *University of Rochester*, 358 F.3d at 925 (considering whether the patent disclosed the compounds necessary to practice the claimed method, given the state of technology); *Singh v. Brake*, 317 F.3d 1334, 1343 [65 USPQ2d 1641] (Fed. Cir. 2002) (affirming adequacy of disclosure by distinguishing precedent in which the selection of a particular species within the claimed genus had involved "highly unpredictable results").

It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. *See In re Angstadt*, 537 F.2d 498, 504 [190 USPQ 214] (CCPA 1976) ("The examples, both operative and inoperative, are the best guidance this art permits, as far as we can conclude from the record"). While the Board is correct that a generic invention requires

adequate support, the sufficiency of the support must be determined in the particular case. Both Eshhar and Capon present not only general teachings of how to select and recombine the DNA, but also specific examples of the production of specified chimeric genes. For example, Eshhar points out that in Example 1 of his specification the FcRg chain was used, which chain was amplified from a human cDNA clone, using the procedure of Kuster, H. et al., J. Biol. Chem., 265:6448-6451 (1990), which is cited in the specification and reports the complete sequence of the FcRg chain. Eshhar's Example 1 also explains the source of the genes that provide the heavy and light chains of the single chain antibody, citing the PhD thesis of Gideon Gross, a co-inventor, which cites a reference providing the complete sequence of the Sp6 light chain gene used to construct the single-chain antibody. Eshhar states that the structure of the Sp6 heavy chain antibody was well known to those of skill in the art and readily accessible on the internet in a database as entry EMBL:MMSP6718. Example 5 at page 54 of the Eshhar specification cites Ravetch et al., J. Exp. Med., 170:481-497 (1989) for the method of producing the CD16a DNA clone that was PCR amplified; this reference published the complete DNA sequence of the CD16a chain, as discussed in paragraph 43 of the Eshhar Declaration. Example 3 of the Eshhar specification uses the DNA of the monoclonal anti-HER2 antibody and states that the N29 hybridoma that produces this antibody was deposited with the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, on August 19, 1992, under Deposit No. CNCM I-1262. It is incorrect to criticize the methods, examples, and referenced prior art of the Eshhar specification as but "a few PCR primers and probes," as does the Director's brief.

Capon's Example 3 provides a detailed description of the creation and expression of single chain antibody fused with T-cell receptor zeta chain, referring to published vectors and procedures. Capon, like Eshhar, describes gene segments and their ligation to form chimeric genes. Although Capon includes fewer specific examples in his specification than does Eshhar, both parties used standard systems of description and identification, as well as

known procedures for selecting, isolating, and linking known DNA segments. Indeed, the Board's repeated observation that the full scope of all of the claims appears to be "enabled"cannot be reconciled with the Board's objection that only a "general plan" to combine unidentified DNA is presented. *See In re Wands*, 858 F.2d 731, 736-37 [8 USPQ2d 1400] (Fed. Cir. 1988) (experimentation to practice invention must not be "undue" for invention to be considered enabled).

The PTO points out that for biochemical processes relating to gene modification, protein expression, and immune response, success is not assured. However, generic inventions are not thereby invalid. Precedent distinguishes among generic inventions that are adequately supported, those that are merely a "wish"or "plan," the words of *Fiers v. Revel*, 984 F.2d at 1171, and those in between, as illustrated by *Noelle v. Lederman*, 355 F.3d at 1350; the facts of the specific case must be evaluated. The Board did not discuss the generic concept that both Capon and Eshhar described — the concept of selecting and combining a gene sequence encoding the variable domain of an antibody and a sequence encoding a lymphocyte activation protein, into a single DNA sequence which, upon expression, allows for immune responses that do not occur in nature. The record does not show this concept to be in the prior art, and includes experimental verification as well as potential variability in the concept.

Whether the inventors demonstrated sufficient generality to support the scope of some or all of their claims, must be determined claim by claim. The Board did not discuss the evidence with respect to the generality of the invention and the significance of the specific examples, instead simply rejecting all the claims for lack of a complete chimeric DNA sequence. As we have discussed, that reasoning is inapt for this case. The Board's position that the patents at issue were merely an "invitation to experiment" did not distinguish among the parties' broad and narrow claims, and further concerns enablement more than written description. *See Adang v. Fischhoff*, 286 F.3d 1346, 1355 [62 USPQ2d 1504] (Fed. Cir. 2002) (enablement involves assessment of whether one of skill in the art could make and use the invention without undue experimentation); *In re Wright*, 999 F.2d 1557, 1561 [27 USPQ2d 1510] (Fed. Cir. 1993) (same). Although the legal criteria of enablement and written description are related and are often

met by the same disclosure, they serve discrete legal requirements.

The predictability or unpredictability of the science is relevant to deciding how much experimental support is required to adequately describe the scope of an invention. Our predecessor court summarized in *In re Storrs*, 245 F.2d 474, 478 [114 USPQ 293] (CCPA 1957) that "[i]t must be borne in mind that, while it is necessary that an applicant for a patent give to the public a complete and adequate disclosure in return for the patent grant, the certainty required of the disclosure is not greater than that which is reasonable, having due regard to the subject matter involved." This aspect may warrant exploration on remand.

In summary, the Board erred in ruling that §112 imposes a *per se* rule requiring

recitation in the specification of the nucleotide sequence of claimed DNA, when that sequence is already known in the field. However, the Board did not explore the support for each of the claims of both parties, in view of the specific examples and general teachings in the specifications and the known science, with application of precedent guiding review of the scope of claims.

We remand for appropriate further proceedings.

VACATED AND REMANDED

Footnotes

1 Although Capon is designated as appellant and Eshhar as cross-appellant, both appealed the Board's decision. *See* Fed. R. App. P. 28(h). The Director of the PTO intervened to support the Board, and has fully participated in this appeal.

- End of Case -